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Harnessing Immune Alterations in Neurodegenerative Diseases

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Immune dysfunction, a well-established feature of neuroinflammatory disease, is increasingly recognized in neurodegenerative conditions. Its role is emerging as an early and active participant in neuropathology. Inflammation could be modified, with disease-slowing effects, by targeted interventions; it is also readily detectible and could serve as a source of valuable biomarkers.

It is hardly controversial to assert that abnormal inflammation can cause damage to the central nervous system. In multiple sclerosis (MS), the quintessential CNS inflammatory disorder, an adaptive immune response of obscure origin, comprising antigen-specific T and B cells, drives acute inflammatory events. Later, an innate immune response (monocytes and microglia) culminates, in progressive cases, in a disease phase that may be more neurodegenerative than inflammatory. The inflammatory component of MS is now well-known to be amenable to modification by interventions targeting the immune system, from broad-spectrum agents such as steroids and interferons to more targeted agents like monoclonal antibodies to individual immune system components. Recently, many have argued that early, aggressive treatment of abnormal immune activity during a key "window of opportunity" may be essential if the neurodegenerative phase of the illness—and its inevitable accumulation of disability—is to be prevented.

Diseases like Alzheimer's (AD), Parkinson's (PD), Huntington's (HD), and amyotrophic lateral sclerosis (ALS) have traditionally been viewed as neurodegenerative, and with good reason: it is primarily the death and dysfunction of neurons that causes disability. But increasingly, activation of various components of the innate immune system is recognized as a feature of these "neurodegenerative" diseases. If the immune system is playing any active role in a neurodegenerative disease, for better or for worse, then modifying it—enhancing favorable behaviors or suppressing harmful ones—may be capable of slowing the progression of the disease. Even if the immune system cannot be manipulated to therapeutic effect, immune derangements could perhaps be harnessed as markers of diagnosis or disease progression.

The peripheral effectors of innate immunity are myeloid-derived blood monocytes that additionally give rise to phagocytic tissue macrophages, and to inflammatory dendritic cells (DC). Conversely, brain microglia (the macrophage/DC of the CNS) are self-renewing and arise from fetal myeloid progenitors. Microglial "activation" is now held to be more complex than an all-or-nothing process. Depending on the stimulus and biochemical milieu, a wide range of qualitatively varied responses—pro- and anti-inflammatory, neuroprotective or neurotoxic—may be elicited. Moreover, microglia experiencing sustained stimulation enter a state of chronic activation in which toxic factors are continuously released, with resulting ongoing damage to surrounding tissue (reviewed in Ransohoff and Perry, 2009).

Microglial activation can be identified histologically and is both widespread and concentrated on areas of pathology in the brains of patients with AD, HD, PD, and ALS (reviewed in Lobsiger and Cleveland, 2007; Ransohoff and Perry, 2009; Schwab and McGeer, 2008). In AD, activated microglia are seen to surround and infiltrate extracellular amyloid beta (Aβ) plaques, while prevention of macrophage and monocyte migration and accumulation results in accelerated pathology. Aβ activates microglia into a proinflammatory state, while simultaneously reducing their phagocytic ability. Overall, while it is clear that microglia are involved in the pathogenesis of AD, it is far from apparent whether their overall role in AD is neuroprotective, pathogenic, or, most likely, a combination of both (Schwab and McGeer, 2008). However, long-term anti-inflammatory drug treatment does appear to reduce susceptibility to AD and PD, and immunomodulatory therapies are currently a major area in neurodegenerative research (Schwab and McGeer, 2008; Hirsch and Hunot, 2009).

Microglial activation appears to be a general response to extracellular fibrillar protein, as it can also be triggered by tau and α-synuclein. Extracellular α-synuclein aggregates, for instance, induce microglial activation and release of proinflammatory cytokines, leading to dopaminergic neurodegeneration in PD (Hirsch and Hunot, 2009). In ALS, activated microglia overexpressing mutant SOD1 (mSOD1) can be seen to contribute to neuronal death, and reducing mSOD1 levels in microglia has been seen to slow disease progression significantly (Boillée et al., 2006). Progression of ALS probably involves the action of abnormal protein within cell types other than motor neurons, which raises the possibility of targeting nonneuronal cells both for

monitoring disease and new therapies (Lobsiger and Cleveland, 2007).

As a penetrant, monogenic condition, HD offers unique insights into immune contributions to neurodegeneration before disease onset. The mutant huntingtin (mHtt) protein is expressed ubiquitously, meaning that the function of neurons, glial cells, and peripheral immune cells may be altered by the mutation. We demonstrated that monocytes/macrophages and microglia are hyperactive in HD, and overexpression of IL-6 and other proinflammatory cytokines can be seen in postmortem HD striatum (Björkqvist et al., 2008). Since microglial activation is known to occur in HD, these findings suggest that not only do microglia in HD find themselves in an abnormal, proinflammatory environment, but they may also respond to this environment maladaptively by potentially detrimental overreaction, due to a direct effect of the mutant htt protein within the microglia (Björkqvist et al., 2008). Indeed, a yeast genomic screen revealed the microglial-specific kynurenine monooxygenase (KMO) pathway to be a highly significant disease modifier in HD (Giorgini et al., 2005), and work is now underway to investigate whether downregulation of microglia or the KMO pathway can alter the natural history of HD.

Clearly an increased understanding of innate immunity appears essential for understanding some neurodegenerative disease pathology. Microglial and innate immune dysfunction may provoke CNS pathogenesis and provide key clinical targets years before disease onset. Alternatively, immune hyperactivity may reflect some of the earliest detectable pathological events, and this may be detectable before disease onset. Therefore, a key question is whether CNS inflammatory activity can be measured in vivo and used for diagnostic purposes as potential biomarkers of progression and/or to measure the efficacy of possible treatments.

The biochemical hallmarks of inflammatory activation, such as increased levels of cytokines and chemokines in the brain, can be detected readily postmortem and in animal models but cannot be directly quantified in vivo in patients. Positron-emission tomography (PET) imaging using the radioligand 11C-(R)-PK11195, which binds the peripheral benzodiazepine-binding site, enables the visualization and quantification of microglial activation in vivo. Using this technique, early microglial activation has been shown in numerous neurodegenerative diseases. In HD, microglial activation can be detected in gene carriers many years prior to disease onset (Tai et al., 2007). Microglial activation can also be detected in patients with mild cognitive impairment (MCI) prior to progression to AD (Fiala and Veerhuis, 2009), further supporting the notion that an innate immune response is an early event in AD, and likewise supporting the importance of microglial activation as a potential biomarker. PET imaging, though expensive and technically challenging, offers one way in which CNS inflammation could be monitored relatively directly as a possible biomarker of neuropathology or response to treatments. However, 11C-(R)-PK11195 cannot readily distinguish between qualitatively different states of microglial activation, so cannot discriminate between helpful and harmful inflammatory activation. Numerous alternative PET ligands that may enable in vivo imaging of other aspects of CNS inflammation are under investigation.

Neuroinflammatory processes in neurodegeneration can also be followed in cerebrospinal fluid (CSF). This has been suggested for PD where an increase in proinflammatory cytokines can be seen in CSF (Hirsch and Hunot, 2009). Likewise, the proinflammatory cytokine TNF-α has been found to be increased in CSF in individuals with MCI who later progress to AD (Fiala and Veerhuis, 2009). Our work in HD suggests there may be parallel derangements of CNS and peripheral inflammatory function that translates into parallel changes in CSF and plasma (Björkqvist et al., 2008). However, CSF levels of most cytokines are unlikely to be useful alone to diagnose or to differentiate different neurodegenerative diseases; instead, CSF inflammatory markers may be a useful tool to monitor anti-inflammatory therapeutic effects.

In MS, plasma immune measures have been shown to be associated with disease activity and MRI activity. However, the neuroinflammation of MS is often striking, widespread, and sustained. Could inflammatory changes detectable in blood inform us about the pathogenesis of primarily neurodegenerative diseases?

Our work in HD suggests that there may be parallel derangements of CNS and peripheral inflammatory function. We found a proinflammatory pattern of cytokine elevation in plasma in HD, with IL-6 significantly elevated in a group of subjects predicted to be, on average, 16 years from the onset of disease signs. Other cytokines including IL-8 and TNF-α were elevated in patients with manifest disease. These changes mirrored the cytokine expression changes seen postmortem in HD striatum. Further experiments using LPS stimulation of monocytes from premanifest HD mutation carriers revealed hyperactivity similar to that seen in HD microglia, arguing in favor of a cell-autonomous effect of mutant huntingtin in peripheral myeloid cells as well as in the CNS. The nature of the interplay between CNS and peripheral inflammatory derangement is unclear, but the passage of inflammatory molecules across the blood-brain barrier, in either direction, with possible effects on the neurodegenerative process, cannot be excluded. Further work exploring the interplay between central and peripheral inflammatory derangement is essential in determining the ability of peripheral innate immune phenotypes to shed light on CNS neurodegeneration (Björkqvist et al., 2008). This is also true of the other neurodegenerative diseases, where the balance of primary and secondary abnormalities of the peripheral immune system has been little studied, but is becoming of increasing interest.

Many studies in AD have reported alterations in systemic immune responses involving both innate and humoral immune systems, including changes in lymphocyte and macrophage distribution and activation, the presence of autoantibodies, complement system alterations, and abnormal cytokine production in cerebrospinal fluid (CSF) and plasma (reviewed in Lucin and Wyss-Coray, 2009 [this issue of *Neuron*]). Increasing evidence from studies in animal models of AD shows that peripheral immune cells infiltrate the brain and may modulate the disease. This work opens up possible new therapeutic avenues in AD

through modulation of peripheral immune function.

The study of the role of microglia in neurodegeneration is limited in part by the inaccessibility of human microglia in vivo. Monocytes and macrophages, as the peripheral immune cell equivalents of central microglia, are easily accessible and may conceivably provide model systems of similar pathological processes occurring in CNS microglia. Experimental assessments of spontaneous and stimulated production of cytokines and chemokines and their receptors in patients with neurodegenerative diseases has been of increasing interest, with cytokine release from blood mononuclear cells in response to stimulation correlating with disease severity in AD (Lucin and Wyss-Coray, 2009). Investigating transcriptional dysregulation in peripheral monocytes in response to stimulation using expression profiling could identify genes specifically dysregulated in disease and may identify gene networks and cellular pathways important for disease pathogenesis and help identify new potential targets for therapeutic intervention.

Study of the peripheral innate immune system may also be relevant to disease symptomatology itself. Weight loss and muscle wasting are commonly found in patients with these disorders, and interestingly there is a potential impact of immune alteration on these aspects of the peripheral phenotype. Similar, depression is a common feature in neurodegenerative diseases, and again there is a potential link to innate immune dysfunction. Therefore, study of peripheral cytokines, chemokines, complement factors, and signaling proteins may provide an accessible source of biomarkers useful in diagnosing neurodegenerative diseases, predicting progression and phenotype, or monitoring therapeutic interventions.

The availability of reliable tests on accessible tissues such as blood would be a valuable asset. As a key modulator of the complement cascade and implicated in neuroinflammation, clusterin was previously shown to be upregulated in peripheral blood in HD (Dalrymple et al., 2007). Recently, large genome-wide association studies have also identified variants in the clusterin gene (*CLUS*) to be associated with an increased risk of sporadic AD (Harold et al., 2009). Clusterin and related proteins have now been implicated in the neuropathogenesis of MS, AD, PD, and AD. Evidently, peripheral inflammatory alterations have the power not only to offer potential biomarkers but also to highlight salient foci of pathogenic significance.

Eighteen proteins in plasma (among them, several proteins involved in the immune response) were also recently shown to classify blinded samples from AD and control subjects with close to 90% accuracy and to identify patients who had mild cognitive impairment that progressed to Alzheimer's disease (Ray et al. 2007). Ongoing inflammatory processes can provide a characteristic profile of immune markers in plasma, creating optimism for finding a detectable disease-specific pattern of changes. In PD, an inflammatory process can also be detected in serum, and high plasma concentrations of IL-6 may predict an increased risk of developing PD (reviewed in Hirsch and Hunot, 2009). In ALS, immune markers can be seen to follow disease progression (reviewed in Turner et al., 2009).

There are, however, strong caveats to the use of plasma cytokines alone as biomarkers of disease progression or diagnostic predictors. It is known that cytokines display diurnal variation and that this can alter with disease. Such disease-related differences make standardization of sampling time challenging. Needless to say, levels of many immune molecules are greatly altered by infection and concomitant inflammatory illness. Other factors likely to influence cytokine levels include immunomodulatory actions of cortisol, body mass index, visceral fat deposits, smoking status, exercise, diet, dietary supplementation (especially fatty acids), and medications, adding to the challenge of developing immune markers specific for disease. Also, neurodegenerative disorders are often slowly progressive, and changes are likely to be subtle and difficult to monitor. More likely perhaps, monocyte function assays or distinct patterns and profiles of changes (Ray et al., 2007) might prove to be useful in following disease progression or as pharmacodynamic biomarkers.

Demonstrating an association between inflammatory markers and neurodegenerative disorders is a necessary first step, but such studies do not themselves establish the full clinical utility of a biomarker. Multicenter longitudinal cohort analysis of candidate markers and trials in which clinical endpoints, possible treatments, and potential biomarkers are studied will be essential in the validation process.

The prospect of immunomodulatory treatments has further ramifications for the development of inflammatory biomarkers, because such therapies would be expected to influence the marker directly. This does not mean that inflammatory markers and treatments are mutually exclusive, but that in the context of such treatments, immune molecules may be pharmacodynamic markers of target occupancy, rather than surrogates for clinical endpoints.

Nonetheless, there is room for optimism that with a better understanding of the innate immune system and its interplay with the CNS in health and disease, and through combining biomarker information from different sources, immune activation could serve as a valuable window into the otherwise hidden CNS milieu.

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